REMARKS

I. Status of the Application

Claims 1-8, 10-15 and 37-39 are presently pending in the application. Claims 40-56 are presented above. Claims 40-56 are identical to the claims presented in the July 19, 2001 amendment except that the claims recite an "array of diverse polymers" and do not further limit the polymers. Applicants wish to provide arguments addressing the Examiner's prior written description and enablement rejections of claims including the phrase "array of diverse polymers". Applicants believe that the phrase meets the written description and enablement requirements of the US patent laws. Applicants have amended the specification to correct minor typographical errors, as suggested by the Examiner.

Claims 1-8, 10-15 and 37-39 stand rejected as anticipated by, or in the alternative, as being obvious over Lam et al. (5,650,489). Claims 1-8, 10-15 and 37-39 stand rejected as being obvious over the Examiner's combination of Lam et al. (5,650,489) in view of Holmes (5,679,773). Applicants respectfully request reconsideration of the application and allowance of the claims.

II. The Written Description and Enablement Rejections In the Previous Final Office Action

At page 2, paragraph 5 of the previous Final Office Action, claims 1-8, 10-15 and 37-39 stood rejected under 35 U.S.C. § 112, first paragraph as lacking adequate written description in the as-filed specification and for lacking enablement as to the claim term "array of diverse

biological polymers". The Examiner stated that the claims encompass a genus that is indefinitely large because the specification only discloses peptide and nucleotide libraries. While the Examiner stated that the specification enables nucleotides, peptides and peptide nucleic acids, the Examiner further stated that an array of diverse polymers was not enabled.

Applicants respectfully request that the Examiner consider the following arguments in support of the patentability of the newly submitted claims 40-56. Applicants respectfully submit that the specification provides adequate written description and enablement for claims reciting a preselected array of diverse polymers. Applicants note that the level of skill in the art of polymer synthesis and labeling is high. Applicants also note that arrays of polymers can be made by sequentially reacting subunits or monomers to make the polymer or by attaching a presynthesized polymer to the substrate. The Examiner's unsupported statement at page 4 of the final office action dated March 22, 2001 that "one has to develop synthetic routes capable of limiting the exact number of monomeric units incorporated into any polymer" is unsubstantiated and does not negate Applicants' clear teachings of monomer by monomer techniques or attaching presynthesized polymers to an array. Applicants respectfully submit that in order to meet the written description and enablement requirements of § 112, Applicants need only teach one of skill in the art how to make and use the invention.

In addition, Applicants claimed invention need not be compatible with every polymer synthesis method known in the art, as suggested by the Examiner at page 4 of the final office action dated March 22, 2001 (where the Examiner suggests without factual support that bulk homogeneous and heterogenous catalysts methods cannot be applied to the present invention).

Monomer by monomer methods, as well as presynthesized polymers, can be used to synthesize a preselected array of diverse polymers as claimed.

Applicants respectfully submit that the specification teaches methods that are not only suited to peptide and oligonucleotide arrays, but also to other polymer arrays (e.g., unnatural arrays) as well. The Examiner has provided no factual basis for the statement at page 4 of the final office action dated March 22, 2001 that the specification fails to give adequate direction and guidance in the preparation of arrays of polymers.

Diverse methods of producing polymer arrays are known in the art and are incorporated by reference in Applicants' specification (see, e.g., page 19, lines 10-19 of the specification). Furthermore, Applicants teach various methods of producing polymer arrays. For example, Applicants teach using a variety of modified monomers that contain two functional groups to support chain elongation. The first group allows the monomer to attach to a growing chain of a polymer, while the second group allows the monomer, after incorporation into a polymer, to attach to the next monomer to be incorporated. Such a synthetic scheme can be adapted to a directed synthesis where the second group is attached to a "protecting group" that serves to block one reactive site of a molecule and allow a chemical reaction to be carried out at another active site (page 16, lines 1-13 of the specification).

Applicants teach methods of blocking functional groups with protecting groups such as photolabile protecting groups (e.g., NVOC or MeNPoc), and chemical protecting groups (e.g., FMOC and DMT). For example, at page 20, lines 10-23 of the specification, Applicants teach a method of producing a polymeric array where a solid support may be modified with linking groups having photolabile protecting groups. Specifically, the photolabile protecting group is

added to the surface of a solid support, and the surface is illuminated through a photolithographic mask (e.g., the protecting group is photodeprotected), thus yielding reactive groups. A monomer containing a photolabile protecting group is then added, which binds to the reactive group attached to the solid support. After photoactivation, a second protected monomer may then be added. The deprotection and coupling cycles may be repeated in order to produce a set of oligonucleotides of a desired length.

The use of deprotection methods to generate unnatural polymers is known in the art. For example, Cho et al. utilized a photolabile deprotection strategy to synthesize an array of oligocarbamates substituted with a variety of sidechains (Science 261, 1303-05 (1993), set forth as exhibit A). The polymers were synthesized from nitrophenyl carbonate monomers bearing the photosensitive protecting group NVOC on a terminal amino moiety. Furthermore, Cho et al. cite several references that teach "efficient methods for the synthesis of unnatural biopolymers." Thus, Applicants' teachings of making polymer arrays combined with the level of skill in polymer synthesis would enable the skilled practitioner to produce a variety of polymer arrays by joining subunits. Such polymers would not only include those constructed from amino acids and nucleotides, but from unnatural subunits as well.

Furthermore, Applicants submit that the specification teaches the synthesis of polymers not only by methods of linking monomeric subunits together, but by a variety of methods. Applicants respectfully direct the Examiner's attention to the specification from page 20, line 30 to page 23, line 9, where Applicants teach that polymers may be synthesized by well-known *in vitro* or *in vivo* methods. An array may then be synthesized by attaching presynthesized polymers using a variety of methods taught in Applicants specification and the references

incorporated therein. Applicants teach numerous art known methods of producing and purifying polymers including solid phase chemistry, various molecular cloning techniques, and *in vitro* methods such as polymerase chain reaction (PCR), ligase chain reaction (LCR), Qβ-replicase amplification (QBR), nucleic acid sequence based amplification (NASBA), strand displacement amplification (SDA), cycling probe amplification reaction (CPR), DNA polymerase reactions, RNA polymerase reactions, and branched DNA polymerase reaction (page 21, line 4, to page 22, line 6 of the specification). These methods, combined with Applicants' teachings of array making, would enable one of skill in the art to generate arrays of polymers of specific sizes on substrates without undue experimentation

Applicants teach various methods of labeling polymers. Labels may be added to polymers by the addition of a labeling linker which contains both a labeling site and a polymer attachment site, adding the label to the surface to which the polymers of the array are attached, and as a monomeric building block of the polymer (page 13, line 1-17, page 33, line 10, to page 38, line 2 of the specification). Methods of incorporating various labels into polymers generated by the *in vivo* and *in vitro* techniques described *supra* are well known and would constitute routine efforts by those of skill in the art. Applicants further teach that suitable polymer labels include ^{32}P, ^{35}S, fluorescent dyes and labels, chromophores, electron dense reagents, enzymes, biotin, dioxigenin, and haptens and proteins for which antiserum is available (page 12, lines 26-30, page 25, lines 12-18, and page 29, line 20, to page 31, line 10 of the specification).

Applicants' teachings, in combination with methods well known in the art, would enable the skilled practitioner to produce a multitude of labeled polymers. For example, polymers may be radiolabeled during synthesis using the various *in vitro* techniques taught in the specification.

More specifically, radioactive molecules such as ^{35}S-methionine and ^{35}S-cysteine may be incorporated into polymers containing amino acids, such as various toxins, venoms, viral epitopes, hormones, lectins, antibodies, and enzymes. Radioactive ^{32}P may be incorporated into phosphorus containing polymers, such as oligonucleotides, during synthesis, and radioactive phosphorus and/or sulfur may be incorporated into various drugs during synthesis as well. Compounds such as drugs, agonists, and antagonists may be labeled with chromophores during chemical synthesis.

In view of the above remarks, Applicants submit that no undue experimentation would be required to make and use the claimed invention. The issue of adequate enablement depends on whether one skilled in the art could practice the claimed invention without undue experimentation. Enablement is not precluded by the necessity for some experimentation such as routine experimentation, even if it is extensive routine experimentation. Given that the specification provides all the guidance necessary to make and/or use the claimed invention, Applicants submit that any experimentation needed to practice the invention, such as the synthesis of polymers, labeled polymers and the preparation of polymer arrays, would be routine. In addition, the fact that experimentation may be complex does not necessarily make it undue, if the art typically engages in such experimentation, if the level of skill in the art is high, or if all the methods needed to practice the claimed invention are well known. *In re Wands*, 8 USPQ2d 1400, 1406 (Fed. Cir. 1988).

> The determination of what constitutes undue experimentation in a given case requires the application of a standard of reasonableness, having due regard for the nature of the invention and the state of the art. (Citations omitted). The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. *Id.* at 1404.

Regarding the Examiner's comments at page 4 of the final office action dated March 22, 2001 that the specification fails to provide working examples that are commensurate in scope with the claimed polymers, Applicants respectfully point out that working examples are not required. Also, the Examiner's statement that the art is inherently unpredictable because predicting *a priori* how to prepare any single polymer cannot be done with certainty is unsupported by any factual support. In addition, this statement fails to recognize that presynthesized or commercially available polymers can be used to synthesize the claimed array. Further, the level of skill in the art of polymer synthesis is high as demonstrated by Cho et al. One of skill in the art would readily understand how to practice the methods of the present invention with respect to diverse polymers without undue experimentation. The fact that Applicants' claimed subject matter is broadly claimed with respect to diverse polymers is not reason by itself to deny patentability. Therefore, given Applicants' teachings and the knowledge in the art, one of skill in the art would be able to practice the invention without undue experimentation and would readily understand that the claimed invention is described with sufficient particularity to demonstrate that Applicants had possession of the claimed invention.

III. Claims 1-8, 10-15 and 37-39 Are Patentable over Lam et al.

At page 3, paragraph 8, of the present Office Action, claims 1-8, 10-15 and 37-39 are rejected as anticipated by, or in the alternative, as being obvious over Lam et al. (5,640,489). Applicants respectfully traverse this rejection. Applicants believe that the following arguments are equally applicable to newly added claims 40-56.

A. Preselected Array

Claim 1, and the dependent claims thereof, are directed to a method of monitoring polymer array synthesis on a solid substrate comprising (i) synthesizing a preselected array of diverse biological polymers connected to cleavable linkers on a solid substrate, whereby the diverse biological polymers occupy different regions of the substrate, (ii) cleaving diverse biological polymers from the solid substrate by cleaving the cleavable linkers, thereby creating a mixture of diverse unbound biological polymers, and (iii) measuring the presence of diverse unbound biological polymers as an indicator of the efficiency of the synthesizing step.

Lam et al. fails to teach or suggest synthesizing a preselected array of diverse biological polymers connected to cleavable linkers on a solid substrate. Applicants submit that the Examiner is not giving due weight to Applicants' recitation of the element ***"preselected array"*** in claim 1, newly presented claim 40, newly presented claim 50 and the dependent claims thereof. Preselected array is defined in the present application, on page 15, lines 25-29, as follows:

> A preselected array of polymers is a spatially defined pattern of polymers on a solid support which is designed before being constructed (i.e. the arrangement of polymers on solid substrate during synthesis is deliberate, and not random).

As discussed in detail below, Lam et al. only teaches single bead-single polymer libraries ***that are random*** and does not teach synthesizing a spatially defined pattern of polymers on a solid support which is designed before being constructed.

Throughout the specification of Lam et al., Lam et al. discloses single bead-single polymer libraries. For example, Lam et al. recites at Column 2, lines 26-29 that "... each solid phase support is attached to a single bio-oligomer species." Thus, each solid phase support of Lam et al. cannot have a preselected array of diverse biological polymers, as recited in claim 1 of the present application.

Lam et al. recites at Column 8, lines 47-50 that "... the amino acids are coupled to substantially all available coupling sites on the solid phase support so that each solid phase support will contain essentially only one species of peptide." Again, each solid phase support of Lam et al. cannot have a preselected array of diverse biological polymers, as recited in claim 1 of the present application, if the solid phase supports of Lam et al. contain essentially one species of peptide.

Lam et al. recites at Column 11, lines 10-13 that "it is to be emphasized that the method of the instant invention allows the synthesis of peptides such that each solid phase support, such as a resin bead, will contain only one species of peptide." Thus, Applicants submit that Lam et al. does not teach or suggest a preselected array of diverse biological polymers connected to cleavable linkers on a solid substrate.

Lam et al. states in Column 39, lines 1-3, that the approach "results in a library of solid phase peptide resin beads wherein each bead comprises only one unique peptide sequence." Lam et al. further states, in Column 39, lines 7-9, that "the one bead-one peptide concept is in

fact of primary importance in the success of the presently disclosed method." Lam et al. discloses, in Column 39, lines 29-32, "as mentioned earlier, the general scheme of the methodology is to synthesize a huge library of random peptides on individual solid phase resin beads such that each resin bead contains a single peptide species." In Column 42, lines 32-35, Lam et al. expressly states that "the ability to synthesize individual peptides on each bead ... is the key to success with the methodology of this present invention." In Column 48, claim 1, Lam et al. recites "wherein a single peptide species is attached to each solid phase support." A single peptide species attached to each solid phase support, as claimed by Lam et al., does not constitute diverse biological polymers connected to cleavable linkers on a solid substrate. Because the beads of Lam et al. only contain a single species of biopolymer, Lam et al. does not teach or suggest a preselected array of diverse biological polymers connected to cleavable linkers on a solid substrate, whereby the diverse biological polymers occupy different regions of the substrate.

The Examiner's argument, on page 8 of the Office Action, that Lam et al. provides motivation to modify the single bead concept fails to consider all the teachings of Lam et al. It is well established that " a reference must be considered in its entirety, i.e. as a whole, including portions that would lead away from the claimed invention." *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540, 220 USPQ 303 (Fed. Cir. 1983), *cert. denied*, 469 U.S. 851 (1984). Lam et al. explicitly provides just such a teaching away when it emphasizes the importance of the single polymer-single bead approach.

The Examiner asserts on page 8 of the Office Action that Lam et al. provides motivation to modify the single polymer-single bead concept to arrange single beads in a spatially defined

pattern because "Lam et al. clearly sets forth the desirability of comparing synthesis protocols". Applicants respectfully submit that there is nothing in the mere desire to compare synthesis protocols that would lead one to arrange the single beads of Lam into a spatially defined pattern. The desirability of comparing synthesis protocols says nothing about the type of substrate, let alone modification of substrates.

The comparison of synthesis protocols would appear to be independent of a single bead or array approach. In any event, the comparison of synthesis protocols in Lam et al. (See Column 34, Example 7) merely disclose comparing the single polymer-single bead concept in Lam et al. with standard solid phase peptide synthesis. Such comparisons provide no disclosure, teaching or suggestion to synthesize a preselected array of diverse biological polymers connected to cleavable linkers on a solid substrate, whereby the diverse biological polymers occupy different regions of the substrate. As discussed above, the disclosure of Lam et al, taken as a whole, teaches synthesis of a single bead-single polymer and teaches away from other approaches.

Also, as discussed above, in Column 42, lines 32-35 of Lam et al., Lam et al, states that "the ability to synthesize individual peptides on each bead ... is the key to success with the methodology of this present invention." Other methods, such as Fodor et al 1991 Science 251:767-773, are disclosed by Lam et al. as being "limited" in the context of the invention of Lam et al. (See col. 3 lines 31-52). At the very least, Lam et al. teaches that methods other than Lam's single polymer-single bead polymer are not desirable. Accordingly, synthesizing a preselected array of diverse polymers in the method of Lam would not be immediately envisioned by one skilled in the art. In fact, Lam counsels against it.

Furthermore, Applicants submit that modification of Lam et al., as suggested by the Examiner, would render Lam et al. unsatisfactory for its intended purpose, as Lam readily states that the single polymer-single bead approach is Lam's "key to success". It is well established that "if proposed modification would render the prior art invention being modified unsatisfactory for its intended purpose, then there is no suggestion or motivation to make the proposed modification." *In re Gordon*, 733 F.2d 900, 221 USPQ 1125 (Fed. Cir. 1984).

B. The Random Arrays of Lam et al.

Lam et al. merely discloses random synthesis of libraries and not synthesis of a preselected array of diverse biological polymers connected to cleavable linkers on a solid substrate, whereby the diverse biological polymers occupy different regions of the substrate, as claimed by Applicants.

Lam et al. discusses random libraries extensively throughout the specification. For example, the title of the Lam et al. reference is "Random Bio-oligomer Library, A Method for the Synthesis Thereof, and a Method of Use Thereof."

In Column 6, lines 24-26, Lam et al. states that "library refers to a collection of substantially random bio-oligomers."

In Column 6, lines 34-37, Lam et al. discloses that "the present invention relates to a method of generating a bio-oligomer library by synthesizing bio-oligomers of random monomer subunit sequences."

In column 6, lines 57-58, Lam et al. recites that a library may be generated by "providing at least two aliquots of a solid phase support for the random subunit sequences."

In Column 7, lines 7-8, Lam et al. discloses that "a random bio-oligomer library may be generated..."

In Column 7, lines 10-11, Lam et al. discloses that "in another embodiment, the random bio-oligomer library may be generated..."

Lam et al. discloses, in column 11, lines 20-22, that "the method may be readily adapted to permit the synthesis of a random peptide pool with 10^5 to 10^9 different peptide species."

In column 12, lines 66-67, Lam et al. discusses incorporation of non-classical amino acids into "the random peptide library."

In Column 14, lines 59-62, Lam et al. discloses random polydeoxynucleotide chain synthesis.

In Column 17, lines 24-26, Lam et al. discloses that "the random bio-oligomer can be reused multiple times."

In Column 19, lines 5-6, Lam et al. discloses that "the cells will be incubated with the random peptide library..."

In Column 21, line 47, Lam et al. discloses use of a matrix "containing a random bio-oligomer library."

In Column 24, lines 12-13, Lam et al. discloses "methods of the present invention for assaying activity of a bio-oligomer of a random library of bio-oligomers..."

In Column 24, lines 37-40, Lam et al. discloses that "X represents random amino acid incorporation."

In Column 28, lines 42-45, Lam et al. discusses therapeutic and diagnostic agents from random bio-oligomer libraries.

In Column 34, lines 66-67, Lam et al. discloses "a library of random tetrapeptides...produced in accordance with Example 6..."

In Column 35, lines 47-50, Lam et al. discusses "the pattern of the library of random peptides produced according to the method..."

In Column 36, lines 8-10, Lam et al. discloses that "the results demonstrate that the random peptide synthesis method of the invention permits the synthesis of a library of random peptides..."

In Column 38, lines 37-41, Lam et al discloses that "instead of relying on a biologic system...to generate a random library, the present methods effectively employ chemical synthesis of huge peptide libraries with each different peptide on an individual bead."

In Column 38, lines 44-47, Lam et al. discloses that "the approach depends on the ability to chemically synthesize a huge random peptide library..."

In Column 38, lines 58-60, Lam et al. discloses that "the peptide library generated by this method will be truly random."

In Column 38, lines 66-67, Lam et al. discloses that "the novel approach for simultaneously synthesizing a vast array of peptides not only provides a truly randomized and equimolar library..."

In Column 39, lines 45-46, Lam et al. discloses that "19 different amino acids were used at each of the five random coupling steps."

In Column 39, lines 29-32, Lam et al. discloses that "as mentioned earlier, the general scheme of the methodology is to synthesize a huge library of random peptides..."

In Column 40, lines 4-5, Lam et al. discloses "identification and selection of specific peptide ligands from the random library..."

In Column 40, lines 19-20, Lam et al. discusses "a random library of peptide beads."

In Column 41, lines 5-8, Lam et al. discloses "a large synthetic random library."

In column 41, lines 57-58, Lam et al. discloses use of "the same random peptide library..."

In Column 43, line 44, Lam et al. discloses a "restricted random library."

In Column 45, line 55, Lam et al. discloses "using a random library..."

In Column 46, line 54, Lam et al. discloses preparation of a "random library of pentapeptides."

In Column 47, lines 23-25 Lam et al. discloses "pentapeptides with the composition tyrosine followed by a random sequence..."

In Column 47, line 32, Lam et al. discloses " a random peptide library."

Thus, Applicants submit that in view of the overwhelming evidence above, one skilled in the art would view the teachings of Lam et al. as directed to synthesis of random libraries.

The Examiner asserts that Lam et al. can be used for synthesis of random libraries as well as synthesis of predetermined sequences. However, even Lam et al.'s predetermined sequences are a library of random bio-oligomers. As evidence of this conclusion, in Column 10, lines 56-60, Lam et al. recites that "this method may be used for the synthesis of random peptides as well as for the synthesis of a peptide library that comprises pre-determined sequences." Example 11 also discusses "A Limited Peptide Library." See Column 43 in Lam et al. Because "library" is defined in Column 6, lines 24-26 of Lam et al. as "a collection of substantially random bio-

oligomers," a peptide library having predetermined sequences must still have substantially random bio-oligomers. That is, even though a portion of the sequence of the bio-oligomer library may be pre-determined, substantially random bio-oligomers must still be present to satisfy the definition of "library" expressly recited in Lam et al. As stated above, a "preselected array of polymers" is defined by Applicants as a spatially defined pattern of polymers on a solid support which is designed before being constructed (i.e. the arrangement of polymers on solid substrate during synthesis is deliberate, and not random). See page 15, lines 25-29 of the specification. Because substantially random bio-oligomers must exist in any library synthesized in Lam et al., Lam et al. does not disclose, teach or suggest synthesizing a preselected array of diverse biological polymers connected to cleavable linkers on a solid substrate, whereby the diverse biological polymers occupy different regions of the substrate.

In any event, even if Lam et al. is viewed as disclosing polymers of predetermined sequence (which it does not), Lam et al. does not teach a spatially defined pattern of polymers on a solid support which is designed before being constructed. Lam et al. simply does not teach or suggest applicants preselected array of diverse polymers in its method.

Applicants respectfully request reconsideration of the application and allowance of the claims

IV. Claims 1-8, 10-15 and 37-39 Are Patentable over Lam et al. in view of Holmes et al.

At page 6, paragraph 9, of the Office Action, claims 1-8, 10-15 and 37-39 are rejected as being unpatentable over Lam et al. (5,640,489) in view of Holmes (5,679,773). Applicants

respectfully traverse this rejection. Lam et al. fails to teach or suggest in its method the step of synthesizing a preselected array of diverse biological polymers connected to cleavable linkers on a solid substrate, whereby the diverse biological polymers occupy different regions of the substrate. In fact, Lam et al. teaches against synthesis methods in its invention that are other than the single polymer-single bead approach.

Holmes is cited for the teaching of the synthesis of polymer arrays on a substrate where each member of the polymer array occupies a different region of the substrate. The Examiner's stated motivation to combine Holmes with Lam et al. to arrive at the claimed method is the disclosure in Holmes at column 19 of "the determination of synthesis fidelity". However, Lam et al. counsel's against the use of methods other than the single-polymer-single bead polymer synthesis approach from which Lam et al. cleaves its polymers. Other methods of making polymers are described by Lam et al. as being "limited". One of skill in the art, therefore, would not use a preselected array of diverse polymers as claimed, since to do so would be contrary to the teachings of Lam et al.

Therefore, Applicants submit that the combination of the references by the Examiner is improper. Applicants respectfully request reconsideration of the application and allowance of the claims.

V. Conclusion

Having addressed all outstanding issues, Applicants respectfully request allowance of the case. To the extent the Examiner believes that it would facilitate allowance of the case, the Examiner is invited to telephone the undersigned at the number below.

Respectfully submitted,

Dated: January 29, 2002

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Version Of Amendments With Markings To Show Changes Made

In the Specification:

Please amend the specification as follows:

At page 1, lines 8-9:

This Application claims the benefit of the U.S. Provisional Application by Barone *et al.* (USSN 60/003726) filed September 13, 1995.

At page 1, lines 19-30:

Methods of forming large arrays of oligonucleotides, peptides and other polymers on a solid substrate are known. Pirrung *et al.*, U.S. Patent No. 5,143,854 (see also PCT Application No. WO 90/15070), McGall *et al.*, USSN 06/440742, Chee *et al.*, SN PCT/US94/12305, and Fodor *et al.*, PCT Publication No. WO 92/10092 describe methods of forming vast arrays of peptides, oligonucleotides and other polymers using, for example, light-directed synthesis techniques.

In the Fodor *et al.* PCT application, methods are described for using computer-controlled systems to direct polymer array synthesis. Using the Fodor approach, one [heterogenous] heterogeneous array of polymers is converted, through simultaneous coupling at multiple reaction sites, into a different [heterogenous] heterogeneous array. *See also*, USSN 07/796,243 and USSN 07/980,523 and Fodor *et al.* (1991) *Science*, 251: 767-777.

At page 2 lines 3-20:

More recently, US applications [SN] USSN 06/440,742, USSN 08/284,064, USSN 08/143,312, USSN 08/082,937 and PCT application (designating the United States) SN PCT/U594/12305, describe methods of making arrays of oligonucleotide and oligonucleotide analogue probes, *e.g.*, to check or determine a partial or complete sequence of a target nucleic acid, or to detect the presence of a nucleic acid containing a specific oligonucleotide sequence. USSN application 08/327,687 and USSN application 06/440,742 describe methods of creating libraries of nucleic acid probes for the analysis of nucleic acid hybridization, and for screening nucleic acid binding molecules, *e.g.*, as potential therapeutic agents.

Additional methods applicable to polymer synthesis on a substrate are described in co-pending Applications USSN 07/980,523, filed November 20, 1992, and USSN 07/796,243, filed November 22, 1991, incorporated herein by reference for all purposes. In the methods disclosed in these applications, reagents are delivered to the substrate by flowing or spotting polymer synthesis reagents on predefined regions of the solid substrate. In each instance, certain activated regions of the substrate are physically separated from other regions when the monomer solutions are delivered to the various reaction sites, *e.g.*, by means of [groves] grooves, wells and the like.

At page 3, lines 9-15:

The present invention provides methods and compositions to monitor the synthesis and coupling of monomers and polymers to solid substrates, *e.g.*, in VLSIPS™ arrays. The methods typically operate by incorporating a detectable label (typically an isomeric label, *e.g.*, as provided by the compositions herein) into the polymers of an array. The polymers are cleaved

from the array, and the efficiency of polymer synthesis assessed by monitoring the detectable label in an appropriate assay.

At page 6, lines 13-21:

In another class of preferred embodiments, the invention provides an array of polymers, such as an array of oligonucleotides or proteins, or non-biological polymers, with a monoisomeric detectable label incorporated into each polymer. For instance, in one embodiment where the array is an oligonucleotide, the invention provides an array of oligonucleotides attached to a solid substrate, wherein the label is a monoisomeric label comprising the structure

wherein F comprises a fluorescent group[.];

At page 7, lines 12-17:

In one preferred group of embodiments, the nucleic acid synthesis reagent has the structure

wherein R_1 is selected from the group consisting of alkyl, aryl, and hydrogen; R_2 is selected from the group consisting of alkyl, and aryl[,]; and FL is a fluorescent moiety.

At page 9, lines 24-26:

R_1 is selected from the group consisting of hydrogen, alkyl and aryl;

R_2 is selected from the group consisting of hydrogen, alkyl and aryl;

R_3 is selected from the group consisting of hydrogen, alkyl and aryl[,];

At page 12, lines 17-25:

The term "capping" in the context of synthesizing an array of polymers refers to a step in which unreacted groups that fail to condense and successfully couple with the next polymer synthesis reagent (*e.g.*, a monomer such as a phosphoramidite or amino acid) are blocked. This insures that subsequent reactions proceed only by propagating chains of desired sequence. For instance, capping typically involves the acetylation of 5'-hydroxyl functions on oligonucleotides. This is accomplished, *e.g.*, using acetic anhydride catalyzed by 4-dimethylaminopyridine (DMAP). Other reagents known to those of skill in the art are also suitable.

At page 14, lines 19-27:

A "nucleic acid" is a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogs of natural nucleotides that function in a manner similar to naturally occurring nucleotides (*See*, copending application USSN 06/440742 for a description of nucleic acid analogues).

An "oligonucleotide" is a nucleic acid polymer composed of two or more nucleotides or nucleotide analogues. An oligonucleotide can be derived from natural sources but is often synthesized chemically. It is of any size. Copending application USSN 06/440742 describes a variety of oligonucleotide analogues.

At page 17, lines 1-26:

support materials include, but are not limited to, glass, polacryloylmorpholide, silica, controlled pore glass (CPG), polystyrene, polystyrene/latex, and carboxyl modified teflon. The solid

substrates are biological, nonbiological, organic, inorganic, or a combination of any of these, existing as particles, strands, precipitates, gels, sheets, tubing, spheres, containers, capillaries, pads, slices, films, plates, slides, *etc.*, depending upon the particular application. In light-directed synthetic techniques, the solid substrate is often planar but optionally takes on alternative surface configurations. For example, the solid substrate optionally contains raised or depressed regions on which synthesis takes place. In some embodiments, the solid substrate is chosen to provide appropriate light-absorbing characteristics. For example, the substrate may be a polymerized Langmuir Blodgett film, functionalized glass, Si, Ge, GaAs, GaP, SiO_2, SiN_4, modified silicon, or any one of a variety of gels or polymers such as (poly)tetrafluoroethylene, (poly)vinylidendifluoride, polystyrene, polycarbonate, or combinations thereof. Other suitable solid substrate materials will be readily apparent to those of skill in the art. Preferably, the surface of the solid substrate will contain reactive groups, such as carboxyl, amino, hydroxyl, thiol, or the like. More preferably, the surface is optically transparent and has surface Si—OH functionalities, such as are found on silica surfaces. A substrate is a material having a rigid or semi-rigid surface. In spotting or flowing VLSIPS™ techniques, at least one surface of the solid substrate is optionally planar, although in many embodiments it is desirable to physically separate synthesis regions for different polymers with, for example, wells, raised regions, etched trenches, or the like. In some embodiments, the substrate itself contains wells, trenches, flow through regions, *etc.* which form all or part of the regions upon which polymer synthesis occurs.

At page 19, lines 20-27:

As described above, diverse methods of making polymer arrays are known; accordingly no attempt is made to describe or catalogue all known methods. For exemplary purposes, light directed VLSIPS™ methods are briefly described below. One of skill will understand that alternate methods of creating polymer arrays, such as spotting and/or flowing reagents over defined regions of a solid substrate, bead based methods and pin-based methods are also known and applicable to the present invention [(*See*, Holmes *et al* (filed 1/17/95) SN 08/374,492)] (*See*, Holmes *et al.* (filed January 17, 1995) USSN 08/374,492).

At page 20, lines 26-30:

As described above, several methods for the synthesis of polymer arrays are [know] known. In preferred embodiments, the polymers are synthesized directly on a solid surface as described above. However, in certain embodiments, it is useful to synthesize the polymers and then couple the polymers to the solid substrate to form the desired array. In these embodiments, polymers are

At page 23, lines 1-9:

described by Beaucage *et*[.] *al.* (Beaucage *et*[.] *al.* (1981) *Tetrahedron Letts.* 22 (20): 1859-1862) prior to attachment on a solid substrate. Bead-based synthetic techniques are described in copending application USSN 07/762,522 (filed September 18, 1991); USSN 07/946,239 (filed September 16, 1992); USSN 08/146,886 (filed November 2, 1993); USSN 07/876, 792 (filed April 29, 1992); PCT/US93/04145 (filed April 28, 1993); and Holmes *et al.* (filed [1-17-95] January 17, 1995) USSN 08/374,492. Finally, as described above, polymers are optionally

synthesized using VLSIPS™ methods in arrays, or optionally cleaved from the array and then reattached to a solid substrate to form a second array.

At page 24, lines 1-18:

optionally protected during polymer synthesis using protecting groups. Among a wide variety of protecting groups which are useful are nitroveratryl (NVOC) α-methylnitroveratryl (Menvoc), allyloxycarbonyl (ALLOC), fluorenylmethoxycarbonyl (FMOC), α-methylnitro-piperonyloxycarbonyl (MeNPOC), -NH-FMOC groups, t-butyl esters, t-butyl ethers, and the like as described, *e.g.*, by Holmes *et al.* (*id*). Various exemplary protecting groups are described in, for example, Atherton *et al.*, (1989) *Solid Phase Peptide Synthesis*, IRL Press, and Greene, *et al.* (1991) *Protective Groups In Organic Chemistry*, 2nd Ed., John Wiley & Sons, New York, NY. The proper selection of protecting groups for a particular synthesis is governed by the overall methods employed in the synthesis. For example, in "light-directed" synthesis, discussed herein, the protecting groups are photolabile protecting groups such as NVOC, MeNPoc, and those described in co-pending Application PCT/U593/10162 (filed October 22, 1993). *See also*, Holmes *et al.* (*supra*); Wang (1976) *J. Org. Chem.* 41: 3258; and Rich, *et al.* (1975) *J. Am. Chem. Soc.* 97: 1575-1579. In other methods, protecting groups are removed chemically, and include groups such as FMOC, di(*p*-methoxyphenyl)phenyl (DMT) and others known to those of skill in the art. *See*, Holmes *et al.* (*supra*).

At page 25, lines 12-29:

In preferred embodiments, the present invention proceeds by labeling polymers in arrays, which are then cleaved from the arrays and analyzed. A variety of labels are appropriate and known. A "label" comprises a moiety which is detectable by spectroscopic, photochemical, biochemical, immunochemical, or chemical means. For example, useful labels include ^{32}P, fluorescent dyes, electron-dense reagents, enzymes ([e.g.] *e.g.*, as commonly used in an ELISA), biotin, dioxigenin, haptens and proteins. In preferred embodiments, the label is detectable spectroscopically, *i.e.*, is chromogenic. Suitable chromogens include molecules and compounds which absorb light in a distinctive range of wavelengths so that a color may be observed, or emit light when irradiated with radiation of a particular wavelength or wavelength range (*e.g.*, a fluorescent label). In preferred embodiments, labels of the present invention have the structure A—B, where A is a detectable moiety, and B is a "linking" or "bridging" group which comprises one or more functional regions which allow the detectable moiety to be incorporated into a polymer, or attached to one end of the polymer, using chemistry similar to that used to connect monomers into the polymer. Examples of suitable bridging regions include alkyl and substituted alkyl carbon chains with 1-30 carbons, or more preferably 3-10 carbons, with functional groups such as oxygen and

At page 28, lines 1-12:

nucleic acid integration element comprising a phosphorous atom[,]; Y is selected from the group consisting of hydrogen, alkyl, or aryl; Y_2 is an alkyl chain from 1 to 30 carbons in length; Z comprises a protecting group; and F comprises a fluorescent group.

In a still more preferred embodiment, the nucleic acid synthesis reagent has the structure

wherein R_1 is selected from the group consisting of alkyl, aryl, and hydrogen; R_2 is selected from the group consisting of hydrogen, alkyl and aryl[,]; and FL is a fluorescent moiety. An example of such a nucleic acid synthesis reagent label is the isomeric nucleic acid synthesis reagent with the structure

At page 30, lines 1-29:

oxacarbocyanine, merocyanine, 3-aminoequilenin, perylene, *bis*-benzoxazole, *bis*-p-oxazolyl benzene, 1,2-benzophenazin, retinol, *bis*-3-aminopyridiium salts, hellebrigenin, tetracycline, sterophenol, benzimidazolylphenylamine, 2-oxo-3-chromen, indole, xanthen, 7-hydroxycoumarin, phenoxazine, calicylate, strophanthidin, porphyrins, triarylmethanes and flavin. Individual fluorescent compounds which have functionalities for linking or which can be modified to incorporate such functionalities include, *e.g.*, dansyl chloride; fluoresceins such as 3,6-dihydroxy-9-phenylxanthhydrol; rhodamineisothiocyanate; N-phenyl 1-amino-8-sulfonatonaphthalene; N-phenyl 2-amino-6-sulfonatonaphthalene; 4-acetamido-4-isothiocyanatostilbene2,2'-disulfonic acid; pyrene-3-sulfonic acid; 2-toluidinonaphthalene-6sulfonate; N-phenyl-N-methyl-2-aminoaphthalene-6-sulfonate; ethidium bromide; stebrine; auromine-0,2-(9'-anthroyl)palmitate; dansyl phosphatidylethanolamine; N,N'-dioctadecyl oxacarbocyanine[:]; NN,N'-dihexyl oxacarbocyanine; merocyanine, 4-(3'pyrenyl)stearate; d-3-aminodesoxy-equilenin; 12-(9'-anthroyl)stearate; 2-methylanthracene; 9-vinylanthracene; 2,2'(vinylene-*p*-phenylene)bisbenzoxazole; *p-bis*(2-(4-methyl-5-phenyl-oxazolyl))benzene; 6-dimethylamino-1,2-benzophenazin; retinol; bis(3'-aminopyridinium) 1,10-decandiyl diiodide; sulfonaphthylhydrazone of hellibrienin; chlorotetracycline; N-(7-dimethylamino-4-methyl-2-

oxo-3chromenyl)maleimide; N-(*p*-(2-benzimidazolyl)-phenyl)maleimide; N-(4-fluoranthyl)maleimide; bis(homovanillic acid); resazarin; 4-chloro-7-nitro-2,1,3-benzooxadiazole; merocyanine 540; resorufin; rose bengal; and 2,4-diphenyl-3(2H)-furanone. Many fluorescent tags are commercially available from SIGMA chemical company (Saint Louis, MO), Molecular Probes, R&D systems (Minneapolis, MN), Pharmacia LKB Biotechnology (Piscataway, NJ), CLONTECH Laboratories, Inc. (Palo Alto, CA), Chem Genes Corp., Aldrich Chemical Company (Milwaukee, WI), Glen Research, Inc., GIBCO BRL Life Technologies, Inc. (Gaithersberg, MD), Fluka Chemica-Biochemika Analytika (Fluka Chemie AG, Buchs, Switzerland), and

At page 31, lines 11-29:

Fluorescent labels are generally preferred, in part because by irradiating a fluorescent label with light, one can obtain a plurality of emissions. Thus, a single label can provide for a plurality of measurable events. Detectable signal may also be provided by chemiluminescent and bioluminescent sources. Chemiluminescent sources include a compound which becomes electronically excited by a chemical reaction and may then emit light which serves as the detectible signal or donates energy to a fluorescent acceptor. A diverse number of families of compounds have been found to provide chemiluminescence under a variety or conditions. One family of compounds is [2,3-dihydro-1,4--phthalazinedione] <u>2,3-dihydro-1,4-phthalazinedione</u>. The most popular compound is luminol, which is the 5-amino compound. Other members of the family include the 5-amino-6,7,8-trimethoxy- and the dimethylamino[ca]benz analog. These compounds can be made to luminesce with alkaline hydrogen peroxide or calcium hypochlorite

and base. Another family of compounds is the 2,4,5-triphenylimidazoles, with lophine as the common name for the parent product. Chemiluminescent analogs include *para*-dimethylamino and -methoxy substituents. Chemiluminescence may also be obtained with oxalates, usually oxalyl active esters, [e.g.] *e.g.*, *p*-nitrophenyl and a peroxide, [e.g.] *e.g.*, hydrogen peroxide, under basic conditions. Other useful chemiluminescent compounds are also known and available, including -N-alkyl

At page 32, lines 21-30:

Accordingly, a preferred embodiment of the present invention utilizes a single optical isomer of all the possible diastereomers of a particular molecule as a label. Methods of purifying diastereomers, and methods of purifying enantiomers used to make diastereomers (i.e., "asymmetric synthesis") are known in the art. March (1992) *Advanced Organic Chemistry: Reactions, Mechanisms and Structure* Fourth Edition, John [Wiely] Wiley and Sons and the references therein, particularly chapter 4, and Lide (ed) *CRC Handbook of Chemistry and Physics* 75th edition and the references therein provide[s] a general guide for the purification of stereoisomers. Briefly, a pair of enantiomers can be separated by reaction with a stereoselective reagent, reactions in the presence of circularly polarized light, or,

At page 38, lines 5-13:

Polymers cleaved from VLSIPS arrays are purified according to a variety of known techniques, including, but not limited to, gel electrophoresis, column chromatography, immunopurification, precipitation, crystallization, dialysis, filtration, high pressure liquid

chromatography (HPLC), flash chromatography, paper chromatography and affinity chromatography. *See, e.g.*, Sambrook, *supra*; Ausubel, *supra*; R. Scopes, *Protein Purification*, Springer-Verlag, N.Y. (1982); Hochuli (1989) *Chemische Industrie* 12:69-70; Hochuli (1990) *"Purification of recombinant proteins with Metal Chelate Absorbent"* in Setlow (ed.) *Genetic Engineering, Principle and Methods* 12:87-98, Plenum Press, N.Y.;

At page 44, lines 11-22:

To [15g] 15 g (88.4 mmol, [1eq] 1 eq) of L-threonine methyl ester hydrochloride in [100mL] 100 mL of dry THF at 0 °C under argon was added, dropwise over [1hr] 1 hr, 265.3 mL (265.3 mmol, 3 eq) of borane-THF ([1M] 1 M). The ice bath was removed and the reaction was stirred at room temperature overnight (18 hr). The solution was cooled to 0 °C and quenched slowly with 180 mL of 10 % acetic acid in methanol. The solution was then evaporated to a brown viscous oil and the oil co-evaporated three times with 100 mL of methanol. The crude material was purified by flash chromatography on silica gel using a step gradient of 1 % to 5 % conc. Ammonium hydroxide in methanol/dichloromethane 3:7 to afford [7.5g] 7.5 g (81 %) of (2S,3R)-2-amino-1,3-butanediol as a viscous oil. This material was dissolved in 50 mL of dry DMF and precipitated with hexanes in the cold to give 1 as a white solid.

At page 45, lines 3-13:

To [5g] 5 g (15.4 mmol, 1 eq) of N-Fmoc-4-aminobutyric acid and 8 mL (46.1 mmol, 3 eq) of dry diisopropylethylamine in 60 mL of dry THF at 0 °C under argon was added 2 mL (16.1 mmol, 1.05 eq) of pivaloyl chloride. The solution was stirred for 1 hr at 0 °C and then

[1.8g] 1.8 g (16.9 mmol, 1.1 eq) of 1 was added in 8 mL of dry DMF. The solution was allowed to warm to room temperature and the solvent removed under vacuum. The oil was dissolved in 100 mL of ethyl acetate and washed with 100 mL of sat. aq. $NaHCO_3$ and 100 mL of brine and dried over anhydrous Na_2SO_4. Filtration and solvent removal gave [7g] 7 g of an oil. The crude product was purified by flash chromatography on silica gel using ethyl acetate/hexanes/1 % triethylamine as eluent to afford [3.4g (53 %)] 3.4 g (53 %) of 2 as a yellow foam.

At page 45, lines 16-24:

To [3.4g] 3.4 g (8.2 mmol, 1 eq) of 2 in 30 mL of dry pyridine under argon at ambient temperature was added [3.1g] 3.1 g (9.1 mmol, 1.1 eq) of 4,-4'-dimethoxytrityl chloride. The reaction was stirred for 18 hr and then the solvent removed under vacuum. The oil was taken up in 50 mL of ethyl acetate and washed twice with 50 mL of saturated aqueous $NaHCO_3$ and 50 mL of brine and dried over anhydrous Na_2SO_4. Filtration and removal of solvent gave about [7g] 7 g of an orange oil. The crude product was purified by flash chromatography on silica gel using ethyl acetate/hexanes 3:2 and 1 % triethylamine as eluent to afford [4.7g] 4.7 g (80 %) of 3 as a white foam.

At page 46, lines 2-10:

The FMOC group was removed by treatment of [4.7g] 4.7 g (6.6 mmol) of 3 with 50 mL of 20 % piperidine in DMF for 2 hr at ambient temperature. The solvent was removed under vacuum to give a white solid. The solid was dissolved in 200 mL of ethyl acetate and washed twice with 100 mL of saturated aqueous $NaHCO_3$ and 100 mL of brine and dried over anhydrous

Na_2SO_4. Filtration and evaporation of the solvent gave a white solid which was purified through a plug of silica gel using ethyl acetate/1 % triethylamine to remove the fulvene followed by elution with 60 % methanol/ethyl acetate/1 % triethylamine to afford [2.5g(89 %)] 2.5 g (89 %) of 4 as a white foam.

At page 46, lines 13-27:

[4g] 4 g (10.6 mmol, 1 eq) of 5-carboxyfluorescein was co-evaporated twice under vacuum with 30 mL of dry pyridine and the mixed with [1.2g] 1.2 g (10.6 mmol, 1 eq) of N-hydroxysuccinimide and [2.3g] 2.3 g (10.6 mmol, 1 eq) of dicyclohexylcarbodiimide in 100 mL of dry THF under argon. The reaction was stirred at ambient temperature for 18 hr and then filtered to remove the insoluble urea. The solvent was removed under vacuum to afford [5g] 5 g of an orange solid. To the crude NHS-ester in 50 mL of 10 % pyridine in dichloromethane was added amine 4 in 20 mL of dichloromethane under argon. The reaction was stirred overnight (18 hr) at ambient temperature. The reaction was poured into 100 mL of brine and the aqueous layer was extracted twice with 100 mL of dichloromethane/isopropyl alcohol 1:1. the organic fractions were combined and dried over anhydrous Na_2SO_4. Filtration and removal of solvent gave an orange solid which was purified by flash chromatography on silica gel using a stepwise gradient of 5 % to 30 % methanol/dichloromethane to afford [7.5g] 7.5 g (83 %) of 5 as a yellow solid.

At page 46, line 30 to page 47, line 8:

To [7.5g] 7.5 g (8.2 mmol, 1 eq) of 5 in 30 mL of dry dichloromethane under argon at ambient temperature was added 12.3 mL (16.4 mmol, 2 eq) of triethylamine and [0.2g] 0.2 g (1.6 mmol, 0.2 eq) of dimethylaminopyridine followed by 6.4 mL (16.4 mmol, 2 eq) of pivaloic anhydride. The reaction was stirred for 20 hr and washed twice with 100 mL of dilute aqueous $NaHCO_3$ (1/10 from saturation) and 100 mL of brine dried over anhydrous Na_2SO_4. Filtration and evaporation of the solvent under vacuum gave a pale yellow foam which was purified by flash chromatography on silica gel using a methanol/dichloromethane/ethyl acetate mixture to afford [5.5g] 5.5 g (66 %) of 6 as a white solid.

At page 48, lines 8-15:

The DMT-on poly-16mer was cleaved automatically from the CPG support on the synthesizer with 2 mL of conc. [$NH_4$0H] NH_4OH into the collection vial containing 50 mL of [1M] 1 M NaOH (final concentration of NaOH is 25 mM), and allowed to stand in the dark at room temperature for [15hr] 15 hr. The solution volume was reduced to about 0.5 mL in a speed-vac. The concentration of the oligonucleotide in A_{495} units (au) per mL was determined by dilution of the crude solution to obtain an absorbance reading between 0.1 au and 1 au/mL. The solution was stored at –20 °C in the dark.

At page 50, lines 1-5:

sealed tightly with a screw-cap and allowed to stand at room temperature for a minimum of 15 hrs. The solution was transferred with a pipetman to a 6 mL glass culture tube and the bottle/chip rinsed twice with 1 mL of sdiH_2O and the rinse portions were added to the tube. The

solution was evaporated to dryness in a speed-vac at medium heat and the residue suspended in 1 mL of sdiH_2O (2 x 0.5 mL rinses).